United States Department of Agriculture
Animal and Plant Health Inspection Service
Biological & Technical Services
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This document is not intended to be a comprehensive listing of all data and permitting requirements for field testing of organisms that produce products intended for pharmaceuticals, Its goal is to provide the public a summary of the confinement measures for organisms being field tested in 2002 (barley, corn, rice, sugarcane, tobacco, and tobacco mosaic virus). Permit conditions are made on a case-by-case basis. The document does highlight other important risk mitigation measures that are addressed by the permitting process.

1. OVERVIEW

APHIS regulations require that measures must be taken that mitigate dissemination of the engineered organism into the environment during movement and while in the receiving facility (laboratory, growth chamber, or greenhouse) as specified in 7 CFR 340. The risk mitigation measures include: (1) adequate identification, packaging and segregation measures to prevent or minimize mixing, spillage and dissemination of viable transgenic plant material, including the flow of fertile transgenic pollen to sexually compatible plants during transit and in the receiving facility; (2) when applicable, methods to minimize the flow of fertile transgenic pollen to other sexually compatible plants within the contained facility or to such plants on the outside; (3) devitalization/disposal of transgenic plant material by suitable means, when no longer in use or authorized. Means of devitalization/disposal could include, but are not limited to, dry heat, steam heat, crushing, deep burial and/or chemical treatment.

For field tests, measures must be taken to confme the transgenic plants to the field site during the defined period of the release and to prevent the transgenic plants or their progeny from persisting in the environment in subsequent growing seasons either within or outside of the site of the confined release. Both the reproductive isolation measures and post-harvest land use restrictions are based on the reproductive biology and seed dormancy characteristics of the species, surrounding land use, and proximity of sexually compatible plants. Additional mitigation measures may be necessary based on the nature of the introduced trait(s) and are made on a case-by-case basis.

During the growing season, measures must be taken to achieve reproductive isolation from plants of the same species and other sexually compatible species that are not part of the confmed release, whether they are cultivated or free-living species. Depending on the plant species, this can be achieved by the use of one or a combination of the following: isolation distance, pollen or pollination-proof caging, netting or bagging of plants prior to flowering, guard rows/ border rows of plants to dilute transgenic pollen, flower removal prior to pollination, use of male sterile lines, use of plant growth regulators to block reproductive development, different flowering time,

and/or termination of the confined field release prior to flowering.

APHIS believes some plants are inappropriate for the production of pharmaceuticals. These plants have characteristics like multiple year seed dormancy (e.g., *Brassica rapa*), are bee-pollinated, and are sexually compatible with weed species in the locality of the field site.

Under the APHIS PPQ permitting system, applicants submit information addressing these mitigation and management issues. APHIS reviews the information and if any the agency deems any additional conditions must be imposed to protect the safety of the environment, these will be part of conditions for granting the permit. For additional information see item 5 below. A sample of permit conditions for a field test of corn is shown in Appendix 1.

2. Summary of confinement measures for organisms producing potential pharmaceuticals for CY 2003

BARLEY

Barley is highly self-pollinating, so outcrossing occurs only rarely. To further minimize any gene flow via pollen and possible physical mixing, applicants will do the following: (1) transgenic barley will be planted no closer than 500 feet from other barley plants; **(2)** transgenic barley will be isolated temporally by sowing the seed no less than 28 days before, or 28 days after any nontransgenic seed that is sown within a zone extending from 500 - 1000 feet away from the transgenic test plot; and (3) the transgenic test plot will be monitored for 2 years following the field test to detect and eliminate any volunteer barley plants.

The regulation for production of foundation seed for nonhybrid barley reflects the rarity of outcrossing in that it requires that the foundation seed barley plants be separated from neighboring barley by only a few feet to allow harvesting machinery to operate so that there is no physical mixing of seed from adjacent barley plots (7 CFR part 201.76). For hybrid barley varieties, the regulations require that this minimal isolation distance be increased to 660 feet because of the reproductive biology of these plants. Nonhybrid barley varieties are male-fertile and shed pollen onto their own stigma before the flower opens, thus effectively precluding cross-pollination (pollination typically occurs about 6-7 weeks after seedling emergence). To produce hybrid barley seed, one of the parents must be male-sterile to allow its flowers to be fertilized by a barley pollen donor plant grown adjacent (hybrid barley seed production has been impractical, so it has not been adopted). The transgenic barley plants will be male-fertile, nonhybrid barley types.

As additional precaution, APHIS has chosen the Confinement protocol described above that uses both a greater isolation distance combined with temporal isolation to ensure lack of gene flow.

CORN

The confmement protocols for all corn field tests far exceed the standards for production of foundation seed. The isolation distance between corn fields for the production of foundation seed corn is 660 feet (200 meters) (7 CFR 201.76).

For all corn protocols:

- Applicants must plant the transgenic corn at sites that are at least 1 mile away from of corn seed production (e.g., breeders, foundation, certified, and registered).

- Applicants must ensure that any corn from previous seasons is harvested and removed in a radius of 0.25 mile of the transgenic corn plot, before the transgenic corn is sown.

- The land within 25 feet of transgenic plant area must remain fallow during the test.

1. OPEN-POLLINATED TRANSGENIC CORN - NO BUFFERS

Applicants must ensure that no other corn plants are grown within a radius of 0.5 miles of the transgenic test plants, at any time during the field test.

Applicants must plant their transgenic corn no less than 21 days before, or 21 days after the planting dates of any other corn that is growing within a zone extending from 0.5 to 1.0 mile of the transgenic test plants.

- With the exception of seed corn production (1 mile isolation described above), there are no other restrictions on corn that is grown at distances more than one mile from the transgenic plants.

2. OPEN-POLLINATED TRANSGENIC CORN - WITH BUFFERS

- Applicants must ensure that no other corn plants, other than those of the buffer strip, are grown within a radius of 0.25 mile of the transgenic test plants at any time during the field test.

- A buffer strip consisting of 6 rows of male-fertile, nontransgenic corn must be planted just inside the 0.25 mile perimeter distance. Buffer plants should be a suitable corn hybrid with synchronous or overlapping pollen shed period relative to the transgenic plants. Buffer plants should be of similar height or taller than the transgenic test plants. Applicants should use confinement and disposal measures which treat buffer strip corn material as if it were transgenic. Buffer strip plants should be destroyed or harvested before the seeds reach maturity to preclude the production of viable seed in these plants.

Applicants must plant their transgenic corn no less than 14 days before, or 14 days after the planting dates of any other corn that is growing within a zone extending from 0.25 to 0.5 mile of the transgenic test plants.

With the exception of seed corn production (1 mile isolation described above), there are no restrictions on corn grown at distances more than 0.5 mile from the transgenic plants.

OPTION 3 - POLLINATION VIA BAGGING OF TRANSGENIC PLANTS AND BORDER ROWS

Comments: These are tests in which movement of transgenic pollen is limited by bags placed over floral structures to control pollination of transgenic or nontransgenic plants located within the "transgenic block."

- Applicants must ensure that tassel bags are placed on the tassels before anthesis.

- After pollination tassels must be removed from the plant and disposed to ensure pollen is not released into the atmosphere

- A **buffer strip consisting of 10 rows** of male-fertile, nontransgenic corn must be planted adjacent to the transgenic plot. Buffer plants should be a suitable corn hybrid with synchronous or overlapping pollen shed period relative to the transgenic plants. Buffer plants should be of similar height or taller than the transgenic test plants. Applicants should use confmement and disposal measures which treat buffer strip corn material as if it were transgenic. Buffer strip plants should be destroyed or harvested before the seeds reach maturity to preclude the production of viable seed in these plants.

- All corn grown within 0.25 miles of test plot will have their silks or female reproductive structures bagged and be temporally isolated by at least 21 days pre or post planting from the transgenic plants. (This isolation requirement does not include corn planted as border rows or unselected isolines. These particular plants will be destroyed).

- Corn between 0.25 miles and 0.4 miles of the plot will be temporally isolated by at least 21 days pre or post planting from the transgenic plants. Applicant controlled corn that has their silks or female reproductive structures bagged will not require time isolation beyond 0.25 miles. Applicant controlled corn planted as border rows around the plot or unselected isolines within the plot, wherein the female reproductive structures are not bagged, will not be time isolated. These particular plants will be destroyed.

- In addition, the transgenic plants must be monitored daily during reproductive phases to ensure effective bagging procedures and confinement of pollen. There are no other restrictions on corn that is grown at distances more than 0.4 mile from transgenic plants

Comment: Beginning in the 2003 growing season, APHIS will discourage the use of confinement

protocols which use buffer strips as a means to reduce required isolation distances. APHIS believes that protocols without buffer strips reduce the difficulties of disposal of plant material and also reduces the chance for inadvertent mixing of transgenic and nontransgenic plant materials.[1]

RICE

To prevent gene flow via pollen, applicants will use border rows of nontransgenic rice to dilute pollen from transgenic rice plants, and a minimal isolation distance from other non-regulated rice of 100 ft, and a temporal isolation of at least 14 days difference in the anticipated flowering period to the closest rice fields outside the 100 feet isolation zone. This distance is 10 times the isolation distance specified for foundation seed under 7 CFR 201.76. Although this isolation distance is significantly lower than corn isolation distance, pollen of cultivated rice loses viability within three to five minutes (OECD, 1999, Consensus Document on the Biology of Oryza sativa (Rice) at http://www1.oecd.org/ehs/ehsmono/12E93640.pdf).

SUGARCANE

Sugarcane is unlikely to flower at the proposed test site. The field shall be monitored every other day for potential onset of flowering during the anticipated period of flowering. To prevent gene flow the reproductive structures, if any, will be bagged during the anticipated flowering season. There are no commercial sugarcane plantings within 10 miles of where the test will take place for gene flow to ever be a concern.

TOBACCO

De-flowering can be an effective method for reducing the chance of pollen escape. When de-flowering is used with transgenic tobacco, the required isolation distance to other tobacco is 1320 feet. If plants are allowed to flower, a distance of 2640 to any other tobacco is required. These distances are substantially longer than the 150 foot distance required for the production of foundation seed (7 CFR 201.76). The field should be visited at least once a week to ensure that plants are not flowering. Transgenic tobacco should not be grown within 1 mile of any tobacco seed production fields.

PLANT VIRUS-BASED TRANSIENT EXPRESSION SYSTEMS IN TOBACCO - TOBACCO MOSAIC VIRUS

Plant viruses are being used as transient expression systems for producing pharmaceuticals in tobacco. In this approach, the plants are not transgenic but the engineered viral genome, upon inoculation of tobacco plants, replicates and produces the desired product in the plant. The

[1] Buffer strips may be acceptable in circumstances when the land is under the direct control of the applicant, and the applicant has submitted to APHIS an acceptable method of disposal, e.g., use as biofuel.

product is then isolated from the virus-infected plant and purified. For more information on this system see case study 3, the side bar at http://www.ostp.gov/html/012201.html.

1) The closest commercial tobacco production site should grow TMV-resistant cultivars.

2) A strip of fallow ground should be maintained around the field of tobacco that is to be infected with the engineered virus and any weeds that are hosts for the virus should be controlled on site by either herbicide application or roguing.

2) A non-host species should be grown in the arable land adjacent to this strip of fallow ground to act as a barrier to the spread of the virus to other fields.

3) Inoculation of the tobacco plants with the virus should be performed by spray applicators that control the distribution of the virus.

4) Harvest of the tobacco is performed with a crosscut mower and the plant material is collected in covered containers for transport to the purification facility.

5) All farm implements that come in contact with infected plants should be cleaned thoroughly to ensure inactivation of any residual engineered virus. The applicant has submitted and APHIS has reviewed data demonstrating the effectiveness of the cleaning procedures.

6) The field site will be redisked at least twice after final harvest to facilitate natural decay of plant material. Additionally, any solid waste plant material resulting from the extraction and purification process should also be plowed into the field. The following year a non-host species should be grown in the field to allow additional time for any remaining engineered virus to biodegrade.

7) Potentially infectious liquid waste from the purification process should be inactivated in accordance with USDA regulation (9 CFR 114.15) and sent to the local waste water treatment facility.

3. POST-HARVEST RESTRICTIONS

Post-harvest land use restrictions may be necessary for a certain number of years following harvest of the transgenic plant material to allow monitoring, removal and destruction of volunteers. Generally, for corn, this would involve monitoring for volunteers either immediately after harvest in warm climates where conditions favorable for germination can be maintained, or in the next growing season in colder climates. Generally, the post-harvest periods used to ensure purity of certified seed may be adapted successfully. For certain plant species, and for certain specific cases, post-harvest land use restrictions may also be necessary for the perimeter of the confined field site itself to monitor for volunteers resulting from potential dissemination of seed, e.g., during mechanical harvesting operations.

Other risk mitigation activities for field tests include:

(A) Adequate identification, packaging and segregation measures to prevent seed mixing, spillage and dispersal into the environment during transit;

(B) Adequate cleaning of seeding and transplanting machinery at the confined field site prior to removal to another location to prevent dissemination of viable transgenic plant material into the environment or machinery used solely for these field tests;

(C) Devitalization/destruction of surplus seed or seedlings, and any viable transgenic plant material remaining after transplantation or after harvesting at the confined field site by suitable means which could include, but are not limited to, dry heat, steam heat, crushing, deep burial, discing into the soil, burning, treatment with appropriately labeled herbicides and/or chemicals (harvested transgenic seed and/or plant material from the confined field site may only be retained in an approved facility if requested at the time of the submission and authorized by the regulatory authority, and should be clearly identified, securely transported, and stored separately from other seed/or plant material to avoid mixing); and

(D) A contingency plan for destruction of viable transgenic plant material in case of accidental release. The plan should include site marking and monitoring to ensure destruction of viable material and immediate notification of regulatory authorities.

4. COMPLIANCE INFRACTIONS

Failure of applicants to submit complete and accurate information for all permit activities may result in a fine of not more than $250,000 or imprisonment for not more than five years or both (18 U.S.C. 1001 APHIS has qualified personnel in every State that can inspect field sites for compliance to the performance standards for field testing. Failure to comply with permit conditions can result in compliance infractions and the applicant can be ordered to take remedial action (7 U.S.C. 7714(b)(1)) if necessary to prevent the spread of plant pests (7CFR 340.4 d 7). In some cases, such as minor infractions where the applicant identifies the infraction, notifies APHIS immediately, and takes prompt and appropriate remedial action, a formal written APHIS response may not be necessary. In other cases, written warnings are issued. For the most serious of infractions, an investigation is conducted by APHIS Investigations and Enforcement Services Staff which usually results in applicants being fined. The applicant can also be assessed a criminal or civil penalty for failing to comply with the regulations (7 U.S.C. 7734). If necessary, to protect the environment or public health, the transgenic organisms can be subjected to the application of remedial measures (including disposal) if determined by the Administrator (7 CFR 340.4 d 7). If the owner fails to take such action, the Department can take the action and recover the cost of the action from the owner (7 U.S.C. 7714(b)(2)).

5. FOR ADDITIONAL INFORMATION:

(A) on permit requirement see http://www.aphis.usda.gov/ppq/biotech/7cfr340.html#340.4
(B) on coordination of review with FDA, see the OSTP's Case Studies of Environmental Regulation for Biotechnology, study 3, the side bar at http://www.ostp.gov/html/012201.html
(C) to request a copy of the User's Guide for Release Permits, please see

http://www.aphis.usda.gov/ppq/biotech/
(D) for electronic copies of environmental assessments, see
http://www.nbiap.vt.edu/cfdocs/fieldtests1.cfm and search for the phenotype - pharmaceutical protein produced
(E) To request information under the Freedom of Information Act (FOIA) see http://foia.aphis.usda.gov/guide/.
(F) For the transcripts of the Plant-Derived Biologics Seminar and Public Hearing on Plant-Derived Biologics, held April 5-6, 2000 in Ames, IA, are now available in PDF format from FDA see http://www.fda.gov/cber/minutes/workshop-min.htm#plant

Appendix 1. ***Sample*** Permit Conditions for APHIS Permits for Corn Plants That Produce Pharmaceuticals

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WITHIN 4 WEEKS AFTER **PLANTING,** please submit the following information for **each site for items 1 thru 6:**

1. A map of the site and global positioning satellite (GPS) coordinates. The coordinates should be for each corner of the plot including border rows (if any).

2. The number of transgenic plants which were actually planted at the test site,

3. The total acreage of the test plot (exclude border rows, if any).

4. A report which indicates the distance fi-om the genetically engineered plants to the nearest maize plant that will be used for food, feed, or seed production. The survey should be done within a distance of **one (1) mile** fi-om the plot of transgenic plants.

5. If your permit allows different containment options, please state the specific containment option(s) used at **each** site. If applicants are using isolation distances to separate open pollinated plants fi-om sexually compatible plants, APHIS encourages applicants to use a laser range finder, It is prudent to allow extra distance as safety measure.

6. Fax the above information to Dr. J. L. White, Branch Chief, at Area Code (301) 734 8669, the Regional Biotechnologist (enclosed), and the State official where the test is being performed (see http://www.aphis.usda.gov/biotech/lt_sta.html for fax numbers).

7. Permits and **Risk** Assessments or a Regional Program Manager (Biotechnology) may conduct an inspection of the test site at the beginning of the test. The permittee is required to notify the State regulatory official, and the appropriate Program Manager (Biotechnology) at least 1-week before the test begins.

8. Additional inspections may be conducted by a Plant Protection and Quarantine Officer. The permittee is required to notify the Regional Program Manager (Biotechnology) and the State Officialat least 1-weekbefore termination of the experiment.

9. A field test data report must be submitted within 6 months after the termination of the field test as described below in 340.4. APHIS views these data reports as critical to our assessment of plant pest risk and development of regulatory policies based on the best scientific evidence. Failure by an applicant to provide data reports in a timely manner for a field trial may result in the withholding of permission by APHIS for future field trials.

10. The test site shall be monitored for any volunteer seedlings for (1) one year after the completion of the harvest of the test plants; if any volunteer seedlings are found, they should be

destroyed before flowering.

11. Biological and Technical Services should be notified of any proposed changes to the protocol referenced in the permit application.

12. This approved Biotechnology Permit (APHIS form #2000) does not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals, including: (1) plants, plant parts or seeds which are under existing Federal or State quarantine or restricted use; (2) experimental use of unregistered chemical; and (3) you may need to consult with FDA or CVB prior to use of genetically engineered crops harvested from the field for experimental uses with animals or humans.

13. These are standard permit conditions mandated in 7 CFR 340.4(f) for all permit activities.

A person who is issued a permit and his/her employees or agents shall comply with the following conditions, and any supplemental conditions which shall be listed on the permit, as deemed by the Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packing material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner so as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit;

(4) The regulated article shall be maintained only in areas and premises specified in the permit;

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article;

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation;

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article;

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the Administrator to be necessary to prevent the spread of plant pests;

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference

number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment;

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the following occurrences:

(i) Orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article;

(ii) In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms);

(11) **A** permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

(i) Import or offer the regulated article for entry only at a port of entry which is designated by an asterisk in 7 CFR 319.37-14(b);

(ii) Notify APHIS promptly upon arrival of any regulated article at a port of entry, of its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose; and iii) Mark and identify the regulated article in accordance with 340.5 of this part.